1655

Attorney Docket 1087.1B(A1-35US3)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: ROBERT J. LIPSHUTZ et al.
Serial No.: 09/519,148
Filed : March 6, 2000

Art Unit : 1655
Examiner : Bradley L. Sisson

Title: INTEGRATED NUCLEIC ACID DIAGNOSTIC DEVICE

COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

TRANSMITTAL LETTER

In response to the Office Action mailed on June 13, 2002, Applicants are transmitting herewith the following documents:

- Response to the Office Action mailed on June 13, 2002
- Petition For One-Month Extension Of Time
- Return Receipt Postcard

Please charge any fees or apply credits to the Deposit Account No. 01-0431. If the enclosed papers are incomplete, please contact the undersigned.

Respectfully submitted,

Ivan D. Zitkovsky, Reg. No. 37,482
6 Freeman Circle
Lexington, MA 02421-7713

Tel. +781-943-4012 (274-8064)
Fax +781-274-8065

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to Commissioner for Patents, Washington, D.C. 20231, on

November 13, 2002

Ivan D. Zitkovsky

#27
CD
11/19/02

Attorney Docket 1087(A1-35US3)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: ROBERT J. LIPSHUTZ et al.
Serial No.: 09/519,148
Filed : March 6, 2000

Art Unit : 1655
Examiner : Bradley L. Sisson

Title : INTEGRATED NUCLEIC ACID DIAGNOSTIC DEVICE

COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

RESPONSE

In response to the Office Action of June 13, 2002, Applicants ask for reconsideration for the following reasons:

In the Office Action mailed June 13, 2002, the Examiner rejected claims 80 through 110 under 35 U.S.C. §103(a) as obvious US Patent 5,304,487 to Wilding et al. in combination with Staecker et al., (Staecker et al. "A Procedure for RT-PCR Amplification of mRNAs on Histological Specimens," BioTechniques, Vol. 16, No. January 1994, pages 76-80) and US Patent 5,587,128 to Wilding et al. The Examiner also rejected claims 111 and 115 under 35 U.S.C. 103(a) as being unpatentable over US Patent 5,304,487 to Wilding et al. and US Patent 5,587,128 to Wilding et al. and Staecker et al., as applied to claims 80-110, 112-114, and 116-124, and further in view of U.S Patent 5,1227,730 to Brelje et al. Applicants respectfully disagree with these rejections for the following reasons:

As claimed in claim 80, for example, the present invention is a method of analyzing a sample in an integrated microfluidic device having at least two chambers in fluid communication. The method includes supplying the sample into a first chamber, performing a first reaction in the first chamber, moving the sample from the first chamber to the second chamber, and performing a second reaction in the second chamber, wherein the second reaction is different from the first reaction, and wherein the first or the second chambers are selected from the group of chambers adapted to perform a preparative reaction, an analysis reaction including hybridization, sample

acquisition, DNA extraction, amplification, IV transcription or labeling. The method also includes performing confocal microscopy on the hybridized sample by detecting an optical signal from the hybridized sample inside of the chamber using a reader device located outside of the chamber; receiving a signal output from the reader device; and analyzing the signal output with a digital computer to indicate a property of the sample based on the confocal microscopy.

As further claimed in claim 110, for example, the microfluidic device includes a probe array immobilized on an internal surface and the recited confocal microscopy is used to detect the hybridized sample on the probe array.

As claimed in claim 106, the method of analyzing a sample in an integrated microfluidic device includes supplying the sample into a first chamber selected from the group consisting of a chamber adapted to perform a preparative reaction, an analysis reaction, sample acquisition, DNA extraction, amplification, IV transcription or labeling. The method also includes moving the sample from the first chamber to a second chamber by employing a valve located in a channel between the first chamber and the second chamber, the second chamber being selected from the group consisting of a chamber adapted to perform a preparative reaction, an analysis reaction, sample acquisition, DNA extraction, amplification, IV transcription or labeling; and receiving a signal output from a reader device and indicating a property of the sample.

The Examiner made **three-reference obviousness** rejections and **four-reference obviousness** rejections. Applicants respectfully submit that, when making these rejections, the Examiner did not establish the *prima facie* case of obviousness as explained in detail below:

Obviousness under 35 U.S.C. §103

The Federal Circuit has restated numerous times the criteria for rejecting a claim under 35 U.S.C. §103 as being obvious. For example, as stated in In re Fritch:

> In proceedings before the Patent and Trademark Office, the Examiner bears the burden of establishing a prima facie case of obviousness based upon the prior art. *In re Piasecki*, 745 F.2d 1468, 1471-72, 223 USPQ 785, 787-88 (Fed. Cir. 1984).

A[The Examiner] can satisfy this burden only by showing some objective teaching in the prior art or that knowledge generally available to on of ordinary skill in the art would lead that individual to combine the relevant teachings of the references. *In re Fine*, 837 F.2d 1071, 1074, 5 USPQ2d 1596, 1598 (Fed. Cir. 1988) (citing *In re Lalu*, 747 F.2d 703, 705, 223 USPQ 1257, 1258 (Fed. Cir. 1988)). The patent applicant may then attack the Examiner's prima facie determination as improperly made out, or the applicant may present objective evidence tending to support a conclusion of nonobviousness. *In re Heldt*, 433 F.2d 808, 811, 167 USPQ 676, 678 (CCPA 1970).
In re Fritch, 23 USPQ2d 1780, 1783 (Fed. Cir. 1992)

Obviousness cannot be established by combing the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination. Under section 103, teachings of references can be combined *only* if there is some suggestion or incentive to do so. *ACS Hosp. Systems, Inc. v. Montefiore Hosp.*, 732 F.2d 1572, 1577, 221 USPQ 929, 933 (Fed. Cir. 1984) Although couched in terms of combining teachings found in the prior art, the same inquiry must be carried out in the context of a purported obvious modification of the prior art. The mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggested the desirability of the modification. *In re Gordon*, 733 F.2d at 902, 221 USPQ at 1127.
In re Fritch, 23 USPQ2d 1780, 1783 (Fed. Cir. 1992), emphasis ours.

Furthermore, MPEP provides the following requirements on the Examiner to establish the *prima facie* case of obviousness:

2143 Basic Requirements of a Prima Facie Case of Obviousness [R-1]

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. MPEP '2143

In the Office Action of June 13, the Examiner stated:

7. Wilding 1 discloses a method for analyzing a sample in an integrated microfluidic device that has a plurality of chambers that are in fluid communication with each other. As seen in column 2, the diameter of the channels can range from 0.1 μm to 500 μm. Said channels are in communication with "fluid handling regions." Said regions are considered to meet the limitation of applicants "at least two chambers." ...

...

8. Column 4, first paragraph, teaches explicitly of the optional use of valves within the fluid communication means.

Applicants respectfully disagree with these statements by the Examiner.

While it is true that the two patents to Wilding teach extraction, purification and a subsequent amplification reaction of the same, these are not performed in two separate chambers separated by a valve. In US Patent 5,587,128, for example in col. 12, Wilding mentions reaction chambers and flow channels of different sizes and cross-sections only generically without specificity. Furthermore, US Patent 5,587,128 describes analytical devices shown in Figs. 6 through 13. For example in connection with Fig. 7 (and similarly Fig. 10), Wilding teaches in col. 21, lines 51 – 59 as follows: "FIG. 7 shows a schematic plan view of a substrate 14 fabricated with a system of flow channels 40 connected via channel 20 to ports 16 and a reaction chamber comprising sections 22A and 22B separated by a flow path 20B. The presence of amplified polynucleotide product in a sample will influence the flow characteristics within the flow channels. The channels 40 in this embodiment are symmetrically disposed and have a progressively narrower diameter towards the center of the pattern." (Emphasis ours) In some places Wilding refers to two reaction chambers (instead of sections 22A and 22B), but still there is no valve located in the flow path between the chambers.

US Patent 5,304,487 describes very similar analytical devices as the 128 patent, suggesting or having a valve only at the input or output ports, but not between the reaction chambers. The Examiner referred to US Patent 5,304,487, col. 4, first paragraph, that recites:

> In one embodiment, the detection region may comprise binding moieties, capable of binding to the analyte to be detected, thereby to enhance and facilitate detection. The detection region also may comprise a fractal region, i.e., a region of serially bifurcating flow channels, sensitive to changes in flow properties of a fluid sample, as is disclosed in U.S. Ser. No. 07/877,701, filed May 1, 1992, the disclosure of which is incorporated herein by reference. The device also may be fabricated with at least three inlet ports, in fluid communication with the flow system, provided with valves, e.g., in an appliance used in combination with the device, for closing and opening the ports to enable the control of fluid flow through the mesoscale flow system.
>
> The mesoscale devices can be adapted to perform a wide range of

> biological tests. Some of the features and benefits of the devices are summarized in Table 1. A device may include two or more separated flow systems, e.g., fed by a common inlet port, with different cell handling chambers in each of the systems to enable two or more analyses to be conducted simultaneously. The devices can be utilized to implement a range of rapid tests, e.g., to detect the presence of a cellular or intracellular component of a fluid sample. The devices may be utilized to detect, e.g., a pathogenic bacteria or virus, or for cell sorting. The invention provides methods and devices for a wide range of possible analysis. Assays may be completed rapidly, and at the conclusion of the assay the chip can be discarded, which advantageously prevents contamination between samples, entombs potentially hazardous materials, and provides inexpensive, microsample analyses. (Col. 4, lines 7 – 40, Emphasis ours)

Therefore, Wilding only teaches the use of a valve in the appliance device or at the input (output) port to his mesoscale system. On the other hand, for example, claim 106 (or claim 107) recites moving the sample from the first chamber to the second chamber by employing a valve located in the channel between the first chamber and the second chamber. In US Patent 5,587,128 or US Patent 5,304,487, Wilding not only does not teach the use of two separate chambers separated by a valve (including the other limitations recited in claim 106 or claim 107), Wilding also does not even hint about the use of a valve located in the flow channel between the chambers. Wilding only uses a valve at the entrance into his device. The publication of Staecker et al. alone or in combination with U.S Patent 5,1227,730 to Brelje et al. does not disclose the method claimed in independent claim 106 or 107 using a valve located in the channel between the first chamber and the second chamber. Therefore, independent claim 106 and independent claim 107 are clearly patentable over prior art of record.

In the Office Action of June 13, 2002, the Examiner also stated:

> Column 3 discloses that the results can be detected through a window, and that such detection includes the use of detectable moieties. Column 9 discloses the optional use of additional components for detecting/viewing the assay results. The aspect that the resultant signal can be viewed through a window is considered to meet the limitation that the "reader" is outside of the chamber (a limitation of independent claims 80 and 93, and claims 81-92, 94-105, 110-124 that depend therefrom).
>
> ...

9. The use of the device in the analysis of nucleic acids, be it DNA or RNA is explicitly taught at column 6. The use of detectable moieties in combination with DNA probes, as in nucleic acid assays, is disclosed at column 7.
10. Wilder 1 does not disclose the use of confocal microscopy; nor the use of electrophoretic separation of nucleic acid fragments.
11. Wilding 2, which is based upon a CIP application that matured into Wilding 1, teaches the use of the device in the analysis of nucleic acids, including the amplification of sequences. The use of arrays in concert with the detection of target sequences is disclosed (column 24).
12. While Wilding 2 does teach the use of readers/detection means that are placed internal to the device, it is also noted that Wilding 2 explicitly teaches that one can detect the signal, e.g., a fluorescent signal, "either visually or by machine, through a transparent window disposed over the detection region."
...
14. Wilding 2 does not teach the use of confocal microscopy.
15. Staecker et al., teach of an assay wherein nucleic acids are subjected to amplification and the resultant amplification product is detected/studied through the use of confocal microscopy.
16. It would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method and device of Wilder 1 and Wilder so to utilize
18. Brelje et al., teach at length of the advantages of performing scanning confocal microscopy, including where nucleic acids are being studied. Table 2, column 10, teaches explicitly of DNA specific stains (Chromomycin A3) as well as the use of fluorescein the same fluorophores used by Staecker et al.
19. It would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Wilder 1, Wilder 2, and Staecker et al., with the method of Brelje et al., so that scanning form of confocal microscopy was used. As set forth in columns 1 and 2, confocal microscopy is well known in the art, yet the aspect of performing scanning confocal microscopy has been found to improve on the design of confocal microscopy. In view of the explicit guidance to use scanning confocal microscopy, and in view of the well-developed nature of confocal microscopy as well as performing nucleic acid assays in integrated microfluidic devices, the ordinary artisan would have been both sufficiently motivated and expectant of success in performing such a combination.

Applicants respectfully disagree with some of these statements by the Examiner. While it is true that US Patent 5,304,487 and US Patent 5,587,128 together suggest detection of a signal through a transparent cover, they do not disclose or suggest the claimed "inside chamber" use of confocal microscopy when combined with the publication Staecker et al.

Applicants respectfully submit that the three-reference combination of the two Wilding patents and the publication Staecker et al. do not disclose all limitations of independent claim 80 or independent claim 93. Furthermore, this three-reference combination does not disclose detection of a hybridized sample on a probe array using confocal microscopy, as recited in dependent claims 110 or dependent claim 114.

As admitted by the Examiner, US Patent 5,304,487 and US Patent 5,587,128 do not disclose or suggest the use of confocal microscopy. There is no teaching in the publication Staecker that would disclose or even suggest the use of confocal microscopy for detecting an optical signal from the hybridized sample inside of the chamber using a device located outside of the chamber. Staecker only teaches the use of confocal microscopy on a slide.

Specifically, on page 76, Staecker discloses the following:

> INTRODUCTION
>
> The nerve growth factor (NGF) family of neurotrophins has been found to play an important role in the innervation of the inner ear of the mouse (4,6); however, thus far a source of NGF production has not been localized in the tissues of the inner ear (6). Attempts to localize NGF production using *in situ* hybridization have been unsuccessful (6) presumably because of the very low copy number of NGF mRNA in this system. We have detected the presence of NGF mRNA in the otocyst-cochleovestibular ganglion complex by RT-PCR and have attempted to apply this technique to localize sites of NGF mRNA expression in the inner ear.
>
> By modifying the method of *in situ* polymerase chain reaction (PCR) on tissue preparations as described by Nuovo (5), we have produced a pool of NGF cDNA by reverse transcription (RT) of mRNA in tissue sections mounted on microscope slides. Following this, PCR amplification of the tissue section with addition of a fifth, fluorescent-labeled deoxynucleotide and a set of NGF-specific primers, allows identification of cells containing NGF cDNA by fluorescent microscopy. The advantages of this method are 1) minute amounts of mRNA can be detected and localized at the cellular level, 2) image analysis of confocal microscopic images allows analysis and a rough estimation of accumulated labeled cDNA and 3) time-consuming autoradiography is avoided. (Emphasis ours)

Thus, Staecker discloses only in situ hybridization and in situ polymerase chain reaction (PCR) but not in situ confocal microscopy; his is confirmed by the following teaching of Staecker. On page 78, Staecker discloses:

After placing the PCR mixture on the specimens, plastic coverslips were placed over the specimen and the surrounding nail polish ring. The slides were placed on the PCR machine block (coy slide cycler, prototype model; Grass Lake, MI, USA). The PCR process was initiated with a time-delay file of 8 min at 82°C. Once the block temperature reached 75°C, the coverslips were lifted and 3 μl of *Taq* buffer containing PCR mixture were added to the slide was covered with mineral oil that had been preheated to 80°C in a separate dry block. Twenty cycles of amplification were run with the following program: an initial denaturing step of 94°C x 2 min, linked to a cycling file of 1) 55°C x 1 min, annealing segment; 2) 72°C x 1 min, extend segment; 3) 92°C x 1 min, denaturing segment; then repeat the cycle of steps 1-3, etc. After the completion of 20 cycles, slides were removed and placed in xylene to remove the mineral oil. The xylene was removed, and the tissue sections were washed 2 times for 20 min each in 0.1 x standard saline citrate (SSC) at 45°C to remove excess fluorescent nucleotides.

ANALYSIS

Slides were examined with either a Zeiss axiophot fluorescent microscope (Carl Zeiss, Thornwood, NY, USA) (450-490-nm wave length fluorescent epilumination) or a Bio-Rad MRC 600 Confocal microscope (Hercules, CA, USA) (Image Analysis Facility, Albert Einstein College of Medicine). Data collection was carried out using a 40x lens and 3x Kallman sampling. The confocal's histogram function was used to compare relative fluorescence per pixel of image, allowing determination of fluorescent staining intensity.

Therefore, Staecker does not disclose or even suggest the use of confocal microscopy for detecting an optical signal from the hybridized sample inside a chamber using a device located outside of the chamber. Moreover, Staecker **teaches away** from this "in situ" use of confocal microscopy. Specifically, after hybridization, Staecker teaches taking the slides out, and then performing confocal microscopy. In other words, Staecker does not teach (or even suggest) performing confocal microscopy on the hybridized sample by detecting an optical signal from the hybridized sample inside of the chamber by a confocal microscope located externally to the chamber. Importantly, none of these references provides any motivation to combine US Patents 5,304,487 and 5,587,128 together with the publication Staecker.

Furthermore, US Patents 5,304,487 and 5,587,128 together with the publication Staecker do not disclose or even detection of a hybridized sample on a probe array using confocal microscopy, as recited in dependent claims 110 or dependent claim 114.

While in U.S Patent 5,1227,730 Brelje discloses scanning confocal imaging system, Brelje does not teach (or even suggest) performing scanning confocal microscopy on the hybridized sample by detecting an optical signal from the hybridized sample inside of a chamber by a confocal microscope located externally to the chamber. Furthermore, Brelje does not teach (or even suggest) performing the scanning confocal microscopy on the hybridized sample on a probe array.

Applicants respectfully submit that, when making the above-mentioned rejections, the Examiner did not establish the *prima facie* case of obviousness. The Examiner did not provide any evidence required to show at least some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to combine the mesoscale systems of Wilding et al (described in US Patents 5,304,487 and 5,587,128) together with the publication Staecker directed to histological specimens on a slide.
Applicants believe that the two devices (i.e., the mesoscale system and a slice support) are not compatible. Furthermore, there is not teaching or suggestion to modify, as required to establish the *prima facie* case of obviousness.

Accordingly, independent claims 80, 93,106 and 107 are clearly patentable over the prior art cited by the Examiner. Dependent claims 81 - 92, 94 – 105, and 108 - 124 include additional novel combination of features. Therefore, all pending claims are in condition for allowance and such action is respectfully requested.

Respectfully submitted,

Ivan D. Zitkovsky, Reg. No. 37,482
6 Freeman Circle
Lexington, MA 02421-7713

Tel. +781-274-8064
Fax +781-274-8065

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to Commissioner for Patents, Washington, D.C. 20231, on

November 13 2002

Ivan D. Zitkovsky